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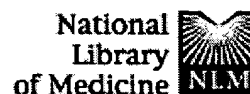
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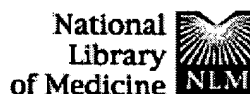
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Thermal hysteresis proteins.

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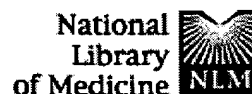
Institute of Biological Sciences, University of Wales, Aberystwyth, Penglais, Ceredigion SY23 3DA, Aberystwyth, UK. jzb@aber.ac.uk

Extreme environments present a wealth of biochemical adaptations. Thermal hysteresis proteins (THPs) have been found in vertebrates, invertebrates, plants, bacteria and fungi and are able to depress the freezing point of water (in the presence of ice crystals) in a non-colligative manner by binding to the surface of nascent ice crystals. The THPs comprise a disparate group of proteins with a variety of tertiary structures and often no common sequence similarities or structural motifs. Different THPs bind to different faces of the ice crystal, and no single mechanism has been proposed to account for THP ice binding affinity and specificity. Experimentally THPs have been used in the cryopreservation of tissues and cells and to induce cold tolerance in freeze susceptible organisms. THPs represent a remarkable example of parallel and convergent evolution with different proteins being adapted for an anti-freeze role.

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Antifreeze and ice nucleator proteins in terrestrial arthropods.

Duman JG.

Department of Biological Sciences, University of Notre Dame, Notre Dame, Indiana 46556, USA. duman.1@nd.edu

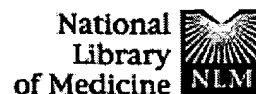
Terrestrial arthropods survive subzero temperatures by becoming either freeze tolerant (survive body fluid freezing) or freeze avoiding (prevent body fluid freezing). Protein ice nucleators (PINs), which limit supercooling and induce freezing, and antifreeze proteins (AFPs), which function to prevent freezing, can have roles in both freeze tolerance and avoidance. Many freeze-tolerant insects produce hemolymph PINs, which induce freezing at high subzero temperatures thereby inhibiting lethal intracellular freezing. Some freeze-tolerant species have AFPs that function as cryoprotectants to prevent freeze damage. Although the mechanism of this cryoprotection is not known, it may involve recrystallization inhibition and perhaps stabilization of the cell membrane. Freeze-avoiding species must prevent inoculative freezing initiated by external ice across the cuticle and extend supercooling abilities. Some insects remove PINs in the winter to promote supercooling, whereas others have selected against surfaces with ice-nucleating abilities on an evolutionary time scale. However, many freeze-avoiding species do have proteins with ice-nucleating activity, and these proteins must be masked in winter. In the beetle *Dendroides canadensis*, AFPs in the hemolymph and gut inhibit ice nucleators. Also, hemolymph AFPs and those associated with the layer of epidermal cells under the cuticle inhibit inoculative freezing. Two different insect AFPs have been characterized. One type from the beetles *D. canadensis* and *Tenebrio molitor* consists of 12- and 13-mer repeating units with disulfide bridges occurring at least every six residues. The spruce budworm AFP lacks regular repeat units. Both have much higher activities than any known AFPs.

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A natural variant of type I antifreeze protein with four ice-binding repeats is a particularly potent antifreeze.

Chao H, Hodges RS, Kay CM, Gauthier SY, Davies PL.

Protein Engineering Network of Centres of Excellence, University of Alberta, Edmonton, Canada.

A 4.3-kDa variant of Type I antifreeze protein (AFP9) was purified from winter flounder serum by size exclusion chromatography and reversed-phase HPLC. By the criteria of mass, amino acid composition, and N-terminal sequences of tryptic peptides, this variant is the posttranslationally modified product of the previously characterized AFP gene 21a. It has 52 amino acids and contains four 11-amino acid repeats, one more than the major serum AFP components. The larger protein is completely alpha-helical at 0 degree C, with a melting temperature of 18 degrees C. It is considerably more active as an antifreeze than the three-repeat winter flounder AFP and the four-repeat yellowtail flounder AFP, both on a molar and a mg/mL basis. Several structural features of the four-repeat winter flounder AFP, including its larger size, additional ice-binding residues, and differences in ice-binding motifs might contribute to its greater activity. Its abundance in flounder serum, together with its potency as an antifreeze, suggest that AFP9 makes a significant contribution to the overall freezing point depression of the host.

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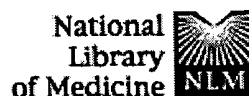
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New ice-binding face for type I antifreeze protein.

Baardsnes J, Kondejewski LH, Hodges RS, Chao H, Kay C, Davies PL.

Department of Biochemistry and the Protein Engineering Network of Centres of Excellence, Queen's University, Kingston, Ont., Canada.

Type I antifreeze protein (AFP) from winter flounder is an alanine-rich, 37 amino acid, single alpha-helix that contains three 11 amino acid repeats (Thr-X(2)-Asx-X(7)), where X is generally Ala. The regularly spaced Thr, Asx and Leu residues lie on one face of the helix and have traditionally been thought to form hydrogen bonds and van der Waals interactions with the ice surface. Recently, substitution experiments have called into question the importance of Leu and Asn for ice-binding. Sequence alignments of five type I AFP isoforms show that Leu and Asn are not well conserved, whereas Ala residues adjacent to the Thr, at right angles to the Leu/Asn-rich face, are completely conserved. To investigate the role of these Ala residues, a series of Ala to Leu steric mutations was made at various points around the helix. All the substituted peptides were fully alpha-helical and remained as monomers in solution. Wild-type activity was retained in A19L and A20L. A17L, where the substitution lies adjacent to the Thr-rich face, had no detectable antifreeze activity. The nearby A21L substitution had 10% wild-type activity and demonstrated weak interactions with the ice surface. We propose a new ice-binding face for type I AFP that encompasses the conserved Ala-rich surface and adjacent Thr.

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